American Journal of Hematology 63:74–78 (2000)

Causes of Venous Thrombosis in Fifty Chinese Patients

Chao-Hung Ho,[1,3]* Wing-Keung Chau,[1,3] Hui-Chi Hsu,[1,3] Jyh-Pyng Gau,[1,3] and Tarng-Jenn Yu[2,3]

[1]Division of Hematology, Department of Medicine, Veterans General Hospital, Taipei, Taiwan, Republic of China
[2]Division of Cardiovascular Surgery, Department of Surgery, Veterans General Hospital, Taipei, Taiwan, Republic of China
[3]National Yang-Ming University, School of Medicine, Taipei, Taiwan, Republic of China

In a whole year from July 1997 to June 1998, a total of 50 patients with sonogram-proved venous thrombosis who called on our hematology clinic consecutively entered into the study. Their mean age was 59.1 ± 17.5 years, range 18–83 years, and 29 were male. A series of examinations were performed in order to find out the cause of venous thrombosis. These examinations included antithrombin, protein C, protein S, plasminogen, heparin cofactor II, activated protein C ratio, factor V Leiden mutation, fibrinogen, factors VIII and XII, euglobulin lysis time, 677 C→T mutation of methylenetetrahydrofolate reductase (MTHFR), prothrombin 20210 (PT 20210) A allele mutation, lupus anticoagulant, anticardiolipin antibody, and complete blood count. Five patients (10%) were found to have malignancy; an inferior vena cava thrombosis in one patient was due to venous compression by hydronephrosis; two patients had lupus anticoagulant; two had varicose veins of legs; two had protein C deficiency; four had protein S deficiency; two had plasminogen deficiency; two had antithrombin deficiency. No activated protein C resistance, elevated factor VIII level, factor V Leiden, PT 20210 A allele or heparin cofactor II deficiency was found in the present study. Homozygous MTHFR 677 C→T gene mutation was found in 7 patients (14%); one of them also had a plasminogen deficiency. No possible risk factor of venous thrombosis could be found in 24 patients (48%). In conclusion, malignancy and protein S deficiency were the most frequent acquired and congenital causes of venous thrombosis in the Chinese, respectively. Am. J. Hematol. 63: 74–78, 2000. © 2000 Wiley-Liss, Inc.

Key words: Chinese population; deep vein thrombosis; etiology; thromboembolism

INTRODUCTION

Thromboembolic diseases (TED) remain a major cause of morbidity and mortality in most countries. More than 80% of the causes of TED had been unknown before 1993. After 1993, APC resistance to factor V Leiden was found to be a major cause of TED in the western countries [1–6], however, the same phenomenon was not found in the Chinese [7,8]. As the Chinese people are less prone to TED [9–11], possibly because of a difference in race, diet or other factors [12–14], the causes of venous thrombosis in the Chinese patients might be different from those of the Caucasians. Thus, investigation of the causes of TED is interested and necessary in the Chinese.

MATERIALS AND METHODS

In a whole year from July 1997 to June 1998, any patients who fitted one of the following criteria were enrolled into the present study: (1) those with recent venous thrombosis proved by sonography; (2) those from hematology out-patient clinic with previously-proved venous thrombosis not more than one year ago, but without investigation of the causes of the thrombosis. In total, fifty patients entered into the study. Their mean age was 59.1 ± 17.5 years, range 18–78 years. Twenty-nine of them were male.

Blood samples were drawn from all patients after signed consent. For the patients with recent thrombosis,

Contract grant sponsor: Veterans General Hospital, Taipei, Taiwan, Republic of China; Contract grant number: 87-60.

*Correspondence to: Chao-Hung Ho, M.D., Division of Hematology, Veterans General Hospital, Taipei, Taiwan, Republic of China.
E-mail: chho@vghtpe.gov.tw

Received for publication 9 April 1999; Accepted 6 October 1999

it was drawn before anticoagulant or thrombolytic therapy, and for those with previous thrombosis, it was drawn after discontinuing all the anticoagulant medications for at least two weeks.

Fasting blood samples from all subjects were obtained to determine antithrombin, protein C, protein S, plasminogen, heparin cofactor II, activated protein C ratio, factor V Leiden, factors VIII and XII, euglobulin lysis time (ELT), 677 C→T mutation of methylenetetrahydrofolate reductase (MTHFR), prothrombin 20210 (PT 20210) A allele, lupus anticoagulant, anticardiolipin antibody, and complete blood count.

Using an automatic instrumentation (STA, Diagnostica Stago, Asnieres, France), we performed antithrombin, protein C, protein S, plasminogen, lupus anticoagulants, and heparin cofactor II assays with the following kits, respectively: STA-Stachrom ATIII, STA-Staclot Protein C, STA-Staclot Protein S, STA-Stachrom Plasminogen, Staclot LA, and Stachrom HCII (all from Diagnostica Stago, Asnieres, France). Protein S and lupus anticoagulants were determined by the clotting method. Antithrombin, heparin cofactor II, protein C, and plasminogen were determined by colorimetric assays. For detailed procedures, see manufacture's instructions.

Using a KC 10A semiautomatic coagulometer (Heinrich Amelung GmbH, Lemgo, Germany), activated protein C resistance, and factor VIII and XII assays were performed with the clotting method with the following reagents, respectively: Coatest APC Resistance (Chromogenix, Molndal, Sweden) and Dade factor VIII or XII deficient plasma (Dade International Inc., FL). For detailed procedures, see manufacturer instructions.

ELT was measured according to Chakrabarti et al. [15]. Complete blood counts were performed using an automated hematology analyzer (Sysmex SE9000, Toa Medical Electronics Co., Ltd., Kobe, Japan).

Anti-cardiolipin antibodies (ACA) were determined by an ELISA technique, using a commercial kit (Anti-CL IgG SELISA, Cambridge Life Sciences, England) for the quantitative detection of ACA of IgG class in human serum or plasma. The Anti-CL IgG SELISA employed a unique antigen-coated microwell technology, utilizing a noncompetitive sandwich enzyme immunoassay system to detect ACA. The testing procedure was conducted according to the manufacturer's instructions.

DNA analysis for the factor V Arg 506–Gln mutation (factor V Leiden), PT 20210A allele, and MTHFR 677C→T mutation were performed with the kit of Coaset FV-506 (Chromogenix, Molndal, Sweden, the method described by Poort et al. [16], and the method described by Frosst et al. [17], respectively. DNA was extracted from the buffy coat of the collected peripheral blood as previously described [18] and stored at 4°C not more than 12 months until analyzed.

Factor V Leiden was detected as previously described [19]. The PCR mixture for analysis of the 20210A allele in the prothrombin gene consisted of 5 pM forward and reverse mutagenic primer [16] in the same buffer and the same PCR conditions as described above. A new *Hin*dIII site (-A/A GCTT-) was introduced in the fragment amplified from the less-frequent allele (A_2:AAG). The more frequent allele (A_1:GAG) lacked the restriction site. The PCR product was then subjected to *Hin*dIII restriction enzyme (New England Biolabs) analysis, and subsequent electrophoresis in 2.5% Metaphor agarose gel (FMC Bioproducts) revealed the mutational status of the subject.

For detection of the 677C→T transition in the MTHFR gene, PCR was performed by use of 5 pM forward and reverse primer [17] in 80 μ*M* dNTPs, 10 m*M* Tris-HCl, pH 8.8 at 25°C, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.1% Triton X-100, and 0.4 U DynaZyme II DNA polymerase, Recombinant (Finnzymes Oy) in a total volume of 50 μL. An initial denaturaiton step was carried out for 5 min at 94°C, followed by 35 cycles of denaturation for 30 sec at 94°C, primer annealing for 50 sec at 57°C, and primer extension for 50 sec at 72°C. A final extension step was performed for 5 min at 72°C and then at 4°C for 30 min. *Hin*fI restriction enzyme (New England Biolabs) analysis and subsequent electrophoresis in a 2.5% MetaPhor agarose gel (FMC Bioproducts) revealed the mutational status of the subject.

Having obtained all the above results and the patients' history, we first analyzed the possible acquired causes which might be related to thrombosis, such as malignancy, nephrotic syndrome, myeloproliferative disorders, antiphospholipid syndrome, post-operation, immobilization, use of the oral contraceptive pill, hormone replacement, or pregnancy. We then rechecked any abnormal levels of each parameter in the remaining patients, analyzed the possible congenital cause of TED in every patient, and calculated the percentage of the congenital abnormality of such parameter to find out the most frequent congenital cause of TED in the Chinese patients.

RESULTS

Possible Acquired Risk Factors in 50 Patients With Venous Thrombosis (Table I)

Forty-seven of the patients proved to have deep vein thrombosis (DVT) of the lower leg, two proved to have superior mesenteric vein thrombosis, and one provided to have deep vein thrombosis of right arm by sonogram. After a series of clinical investigations, neither nephrotic syndrome, polycythemia vera, post-operation, immobilization, use of the oral contraceptive pill, hormone replacement, nor pregnancy could be found. The mean factor VIII level was 0.92 ± 0.21 U/mL, most of the patients had factor VIII level <1.2 U/mL, only one of them >1.2 U/mL (1.26 U/mL). Five of the 50 patients (10%) had malignant disease: one proved to have chronic myelog-

TABLE I. Possible Risk Factors of Thrombosis in 50 Patients With Venous Thrombosis

Risk factor	No of patients		
	Single risk factor	Associated with other risk factor	Total
Malignancy	5	0	5
Venous compression by benign cause	1	0	1
Lupus anticoagulant	2	0	2
Varicose vein of both legs	2	0	2
Protein C deficiency	2	0	2
Protein S deficiency	4	0	4
Plasminogen deficiency	1	1[a]	2
Antithrombin deficiency	2	0	2
Homozygous MTHFR gene mutation	6	1[b]	7
Idiopathic			24

[a]Associated with homozygous MTHFR gene mutation
[b]Associated with plasminogen deficiency

enous leukemia, one had follicular malignant lymphoma, one had gastric adenocarcinoma with metastasis, one had adenocarcinoma of lung, and one had metastatic adenocarcinoma with unknown origin and chronic DIC. Five patients were thought to have benign causes: one had hydronephrosis with compression to inferior vena cava causing venous thrombosis, two were found to have severe varicose veins of lower leg, and two proved to have lupus anticoagulant.

Possible Congenital Risk Factors in 50 Patients With Venous Thrombosis (Table I)

Activated protein C resistance, factor V Leiden, PT 20210A allele or deficiency of heparin cofactor II was not found in our 50 patients with venous thrombosis. Seven patients had homozygous MTHFR gene mutation, one of which was also associated with plasminogen deficiency. Protein S deficiency was found in 4 patients, and antithrombin, protein C, and plasminogen deficiency were each found in two patients, respectively. One of the patients with plasminogen deficiency also had the homozygous MTHFR gene mutation. Finally, no definite known abnormality could be found in 24 patients (48%).

DISCUSSION

TED occurs more frequently in the westerners than in Chinese [9–11]. During the past few years, much effort has been made to better understand the causes of idiopathic venous thrombosis, i.e. the discoveries of factor V Leiden, PT 20210A allele, MTHFR gene mutation and elevated factor VIII levels [1–6,17,18,20,21]. However, though factor V Leiden was found to be the most frequent cause of TED in the western countries, it was absent in the Chinese race [7,8]. Thus, the cause of TED in the Chinese is probably different and deserves our investigation.

Fifty patients with sonogram-provided venous thrombosis who called on our hematology clinic consecutively entered into our study over a one-year period. A series of examinations, including almost all the recently known possible causes of venous thrombosis, were made of these patients to check the possible causes of venous thrombosis. For the possible acquired risk factors of TED, we found that 5 (10%) had malignancy at the time of admission, i.e., their first manifestation of malignancy was deep vein thrombosis. This incidence is much higher than the reports by the Danish group of 2.5% (390/15348, in one year) [22] and by the Swedish group of 4% [23]. Both the Danish and the Swedish studies were from their national registry and thus were retrospective observations. According to Buller et al. [24], the hospital-discharge codes could not exclude patients with a previous bout of venous thromboembolism or a family history, which would indicate an inherited thrombotic disorder rather than primary venous thromboembolism. In addition, patients in whom cancer was found during the initial hospitalization for the thromboembolic event were likely to have been missed in the computerized search of hospital records. In the western countries, the most possible cause for thrombosis is factor V Leiden, and the incidence of thrombosis is much higher than that of the Chinese. Thus, the cause of malignancy in the western countries will be diluted by factor V Leiden or other causes; in the Chinese, however, factor V Leiden is not present as a cause of thrombosis, and thus malignancy is more likely to become an important risk factor of thrombosis. Evaluation of the possibility of cancer in the patients with venous thrombosis is necessary in the Chinese people as the incidence of malignancy in venous thrombosis is not low.

Polycythemia vera was not found as a cause in the present study. This is different from the Swedish report [23] that polycythemia vera had a high standardized incidence ratio (12.9). In our experiences, the incidence of TED in the Chinese patients with polycythemia vera was very low. Thus, polycythemia is not likely to be an important cause of TED in the Chinese.

Elevated factor VIII:C level (>1.5 U/mL) was suggested as the single commonest abnormality detected in 66 of 260 thrombotic patients (25.4%) in the study group of O'Donnell et al. [21]. The prevalence of elevated factor VIII level in the Chinese patients with thrombosis has not yet been reported. In the present study, though factor VIII might be an acute-phase reactant, elevated factor VIII level could not be found in our patients. The highest factor VIII in our study was 1.26 U/mL which occurred in only one patient. All other patients had factor VIII level <1.2 U/mL. Thus, factor VIII seemed not to be a risk factor of thrombosis in the Chinese patients.

Two patients with lupus anticoagulant and two patients with varicose veins were found in the study. The association of lupus anticoagulant and thrombosis was well-documented [25–27], thus, the presence of lupus anticoagulant is a likely cause of thrombosis of this patient. On the other hand, the association with venous thrombosis and varicose veins might need further investigation [28]. However, since a local hypercoagulable state could be detected in a certain proportion of patients with venous stasis [29], this makes varicose veins a probable cause of lower leg venous thrombosis.

Deficiency of plasminogen, protein C, protein S, and antithrombin could be found in totally ten patients (20%) in the present study. The most frequent type of deficiency in our study was protein S deficiency (8%), followed by antithrombin, protein C, and plasminogen (4% each). In our study, we did not investigate the cause of low protein S, i.e., whether it was due to high C4b binding protein or low free protein S and/or total protein S. Further studies should be performed in order to reach a conclusion. The present result is quite similar to another report that protein deficiency is the more frequent cause of venous thrombosis in the Chinese [30]. In that report, the prevalence of protein deficiency was found to be very high (58.8%). Bertina reported that proteins C, protein S, and antithrombin deficiencies comprised about 15% of families with inherited thrombophilia [31]. Families with thrombophilia are highly selected and are expected to show a much higher level of inherited contributing factors than the more or less consecutive thrombosis patients that were studied in the present study. In consideration of the relatively higher contribution of non-factor V Leiden, non-PT20210A allele, and normal factor VIII status in the Chinese, and the reported higher prevalence protein deficiency rates in the Chinese by Shen et al. [30], a higher prevalence of protein deficiency in the Chinese is possible. The higher prevalence rate reported by Shen et al. was probably due to the selected effect of their cases. However, in both studies, the most frequently seen protein deficiency in the Chinese was protein S, not protein C as in the western countries [32].

Plasminogen deficiency was thought to be the risk factor of thrombosis, though Dolan and Preston reported that the association of plasminogen deficiency and thrombosis was not very strong [33]. In our present study, two patients were found to have plasminogen deficiency.

Though heparin cofactor II deficiency was not commonly seen, its deficiency could induce thromboembolic episodes [34]. None of the patients was found to have heparin cofactor II deficiency in our study. Although without sufficient data, we might infer that heparin cofactor II deficiency is not a common cause of thrombosis in the Chinese.

Activated protein C resistance, factor V Leiden, and PT 20210A allele were not found in the present study. DNA analysis has been performed in another study in more than 1261 Chinese subjects to find none with factor V Leiden or PT 20210 A allele [19]. Therefore, we suspected that these two mutations were not present and were thus not the causes of venous thrombosis in the Chinese. It is similar to the report of Lin et al. that PT20210 is extremely rare in the Chinese [35].

Seven patients (14%) were found to have homozygous MTHFR gene mutation. One of the main functions of MTHFR is remethylation of homocysteine to methionine [36]. A common MTHFR mutation, analanine-to-valine substitution, renders the enzyme thermolabile leading to elevated plasma levels of the amino acid homocysteine [37], which was proved to be a risk of occlusive vascular disease [38]. Although hyperhomocysteinemia was reported to be associated with arterial and venous thrombosis [39,40], the association of the MTHFR mutation and thrombosis was controversial. Some studies showed that there was no association between the MTHFR gene mutation and the risk of coronary artery disease or venous thrombosis [41–43]. Others reported positive association between them [44,45]. In our study, since we did not perform plasma homocysteine concentration in our patients, we cannot conclude whether the homozygous MTHFR gene mutation is a risk factor of TED or not. Thus, if we regarded the homozygous MTHFR gene mutation as a risk factor of thrombosis in the Chinese, no cause could be found in 24 of the 50 (48%) patients; if not, 30 (60%) of our patients would have no causes of thrombosis. From these results, we conclude that at least about 40% of the cause of venous thrombosis could be found in the Chinese.

In conclusion, malignancy and protein S deficiency are the most frequent acquired and congenital risk factors of venous thrombosis in the Chinese. Thus, it is necessary to search for hidden cancers in Chinese patients with venous thrombosis. Factor V Leiden, PT 20210 A allele, polycythemia vera, elevated factor VIII level, and heparin cofactor II deficiency were not found as the causes of thrombosis in the present study. Whether MTHFR gene mutation plays a role in formation of venous thrombosis in the Chinese deserves further investigations.

REFERENCES

1. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: Prediction of a cofactor to activated protein C. Proc Natl Acad Sci 1993;90:1004–1008.
2. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–67.
3. Dahlback B. Resistance to APC, the Arg^{506} to Gln mutation in the factor V gene, and venous thrombosis. Thromb Haemostas 1995;73: 739–742.

4. Hajjar KA. Factor V Leiden—An unselfish gene? N Engl J Med 1994; 331:1585–1587.
5. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346:1133–1134.
6. Dahlback B. Molecular genetics of venous thromboembolism. Ann Med 1995;27:187–192.
7. Ho CH. Prevalence of activated protein C resistance in the Chinese population. Thromb Res 1997;88:409–412.
8. Chan LC, Bourke C, Lam CK, Liu HW, Brookes S, Jenkins V, Pasi J. Lack of activated protein C resistance in healthy Hong Kong Chinese blood donors—Correlation with absence of Arg506-Gln mutation of factor V gene. Thromb Haemostas 1996;75:522–523.
9. Chan CW, Hoaglund FT. Pulmonary thromboembolism and venous thrombosis in Chinese. Clin Orthop 1980;50:253–260.
10. Cheng KK, Lai ST, Yu TJ, Duo SM. Postoperative deep vein thrombosis in the Taiwanese Chinese population. Am J Surg 1987;153:302–305.
11. Woo KS, Tse LKK, Tse CY, Metreweli C, Vallance-Owen J. The prevalence and pattern of pulmonary thromboembolism in the Chinese in Hong Kong. Int J Cardiol 1988;20:373–380.
12. Ho CH, Jap TS. Fibrinolytic activity in Chinese patients with diabetes or hyperlipidemia in comparison with healthy controls. Thromb Haemostas 1991;65:3–6.
13. Ho CH, Jap TS. Relationship of plasminogen activator inhibitor-1 with plasma insulin, glucose, triglyceride and cholesterol in Chinese patients with diabetes. Thromb Res 1993;69:271–277.
14. Ho CH, Chwang LC, Hwang BH. The influence of race on the fibrinolytic activity. J Med Sci 1993;13:349–354.
15. Chakrabarti M, Bielawiec JF, Evans JF, Fearnley GR. Methodological study and a recommended technique for determining the euglobulin lysis time. J Clin Pathol 1968;21:698–701.
16. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698–3703.
17. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10: 111–113.
18. Buffone GJ, Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. Clin Chem 1985;31:164–165.
19. Ho CH, Chau WK, Hsu HC, Gau JP, Chih CM. Prevalence of factor V Leiden in the Chinese population. Chin Med J (Taipei) (in press).
20. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995;345:152–155.
21. O'Donnell J, Tuddenham EGD, Manning R, Kemball-Cook G, Johnson D, Laffan M. High prevalence of elevated factor VIII levels in patients referred for thrombophilia Screening: role of increased synthesis and relationship to the acute phase reaction. Thromb Haemostas 1997;77:825–828.
22. Sorensen HT, Mellemkjar L, Steffensen FH, Olsen JH, Nielsen GL. The risk of a diagnosis of cancer after primary deep venous thrombosis or pulmonary embolism. New Engl J Med 1998;338:1169–1173.
23. Baron JA, Gridley G, Weiderpass E, Nyren O, Linet M. Venous thromboembolism and cancer. Lancet 1998;351:1077–1080.
24. Buller H, TenCate JW. Primary venous thromboembolism and cancer screening. New Engl J Med 1998;338:1221–1222.
25. Much JR, Herbst KD, Rapaport SI. Thrombosis in patients with the lupus anticoagulant. Ann Intern Med 1980;92:156–159.
26. Boey ML, Colaco CB, Gharavi A, Elkon K, Liozou S, Hughes GRV. Thrombosis in systemic lupus erythematosus: Striking association with the present of circulation lupus anticoagulant. Br Med J 1983; 287:1021–1023.
27. Triplett DA. Antiphospholipid antibodies and thrombosis. A consequence, coincidence or cause? Arch Pathol Lab Med 1993;117:78–88.
28. Kroger K, Rudofsky G. Duplex sonography of venous stasis. Angiology 1997;48:523–527.
29. Ikeda M, Kambayashi J, Iwamoto S, Shinoki N, Nakamura T, Shibuya T, Kawasaki T, Monden M. The coagulofibrinolytic state of patients with primary varicose veins of the lower legs. Surg Today 1996;26: 985–989.
30. Shen MC, Lin JS, Tsay W. High prevalence of antithrombin III, protein C and protein S deficiency, but no factor V Leiden mutation in venous thrombophilic Chinese patients in Taiwan. Thromb Res 1997; 87:377–385.
31. Bertina RM. Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. Clin Chem 1997;43:1678–1683.
32. Allaart CF, Briet E. Familial venous thrombophilia. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, editors. Haemostasis and thrombosis. Edinburgh: Churchill Livingstone; 1994. 1349–1360.
33. Dolan G, Preston FE. Familial plasminogen deficiency and thromboembolism. Fibrinolysis 1988;2(Suppl 2):26–34.
34. Vinazzer H, Stocker K. Heparin cofactor II: Experimental approach to a new assay and clinical results. Thromb Res 1991;61:235–241.
35. Lin JS, Shen MC, Tsay W. The mutation at position 20210 in the 3′-untranslated region of the prothrombin gene is extremely rare in Taiwanese Chinese patients with venous thrombophilia [letter]. Thromb Haemostas 1998;80:343.
36. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1991;55:131–138.
37. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
38. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–1057.
39. Schwartz SM, Siscovick DS, Malinow MR, Rosendaal FR, Beverly RK, Hess DL, Psaty BM, Longstreth WTJ, Koepsell TD, Raghunathan TE, Reitsma PH. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 1997;96:412–417.
40. Gaussem P, Siguret V, Aiach M. Evaluation of hemostasis in venous thromboembolism pathology (in French). Ann Biol—Paris 1998;56: 49–56.
41. Reinhardt D, Sigusch HH, Vogt SF, Farker K, Muller S, Hoffmann A. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. Eur J Clin Invest 1998;28:20–23.
42. Verhoef P, Rimm EB, Hunter DJ, Chen J, Willett WC, Kelsey K, Stampfer MJ. A common mutation in the methylenetetrahydrofolate reductase gene and risk of coronary heart disease: results among U.S. men. J Am Coll Cardiol 1998;32:353–359.
43. Tosetto A, Missiaglia E, Frezzato M, Rodeghiero F. The VITA project: C677T mutation I the methylene-tetrahydrofolate reductase gene and risk of venous thromboembolism. Br J Haemotal 1997;97:804–806.
44. Cattaneo M, Tsai MY, Bucciarelli P, Taioli E, Zighetti ML, Begnell M, Mannucci PM. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (factor V:Q506). Arterioscl Thromb Vas 1997;17:1662–1666.
45. Kluijtmans LA, van Den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, den Heijer M, Trijbels FJ, Rozen R, Blom HJ. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996;58:17–20.